Specificity of Coproporphyrinogen Oxidase: Conversion of Coproporphyrinogen-IV into Protoporphyrin-XIII

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Summary Coproporphyrinogen-IV (**1b**) is converted into protoporphyrin-XIII by avian haemolysates, and the structure of the product confirmed by total synthesis.

WE have shown[1] how coproporphyrinogen-III (**1a**) is converted by avian haemolysates and rat liver homogenates by a specific pathway into protoporphyrin-IX. Coproporphyrinogen-IV (**1b**) is also a substrate for the enzyme involved, coproporphyrinogen oxidase [EC1.3.3.3], whereas coproporphyrinogens-I and -II are not.[2]

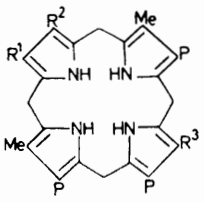
There is indirect evidence[3] that pentacarboxylate porphyrinogen-III (**1c**) is also a substrate for this enzyme, whereas the product of this reaction, dehydroisocoproporphyrinogen (**2c**) is not. Comparison of the structures of these six compounds suggests that the minimum structural requirement necessary for a substrate is the sequence [methyl† methyl-propionate methyl]. We therefore reasoned that the product from coproporphyrinogen-IV would be protoporphyrin-XIII (**2b**).‡

Coproporphyrinogen-IV (*ca.* 2 mg) was shaken with a haemolysate[5] of chicken erythrocytes at 37 °C for 3 h in the dark and the product (*ca.* 1 mg) after isolation in the usual way was converted into the methyl ester. T.l.c. and h.p.l.c.[6] showed that it was essentially a single compound with two ester side-chains. Field desorption and electron impact mass spectrometry gave a molecular ion (*m/e* 590) corresponding to a protoporphyrin dimethyl ester, and n.m.r. spectroscopy in the presence of the shift reagent

† Can be replaced by H or vinyl.

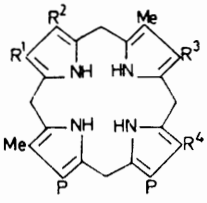
‡ Professor B. Frydman (Buenos Aires) independently reached the same conclusions.

[Eu(fod)$_3$] showed that the two remaining ester groups flanked one *meso*-position (as the corresponding *meso*-proton experienced a very marked downfield shift[7]). Of the



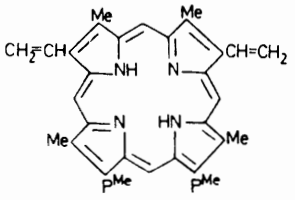
(1)

a; $R^1 = R^3 = Me$, $R^2 = P$
b; $R^1 = P$, $R^2 = R^3 = Me$
c; $R^1 = Me$, $R^2 = P$, $R^3 = CH_2CO_2H$



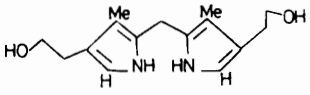
(2)

a; $R^1 = R^4 = Me$, $R^2 = R^3 = CH{=}CH_2$
b; $R^2 = R^4 = Me$, $R^1 = R^3 = CH{=}CH_2$
c; $R^1 = Me$, $R^2 = CH{=}CH_2$, $R^3 = P$, $R^4 = CH_2CO_2H$

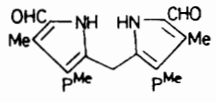


(3)

$P = CH_2CH_2CO_2H$



(4)



(5)

$P^{Me} = CH_2CH_2CO_2Me$

four possible protoporphyrin isomers which might have been formed from coproporphyrinogen-IV (by oxidative decarboxylation of two propionate groups) only one, the XIII isomer (**3**), retains this arrangement of propionate ester groups.

Protoporphyrin-XIII dimethyl ester (**3**) was synthesised from the two pyrromethanes (**4**) and (**5**) followed by conversion[8] of the hydroxyethyl side-chains into vinyl groups The product (**3**), m.p. 198—200 °C, was identical with that obtained from coproporphyrinogen-IV by mixed m.p. and 'mixed' n.m.r. spectra in the presence of the europium shift reagent;[9] *e.g.* the *meso* protons gave rise to a 1:2:1 triplet at τ *ca.* −0·2 to −0·4 (a 3:1 doublet at low concentration) and on addition of shift reagent (5 mol. equiv.) a singlet moved downfield to τ *ca.* −6 whereas the other signals were relatively little affected.

Studies with the enzyme present in liver homogenates show that an intermediate tricarboxylic porphyrinogen is formed in appreciable amounts (*ca.* 35%) *en route* to protoporphyrin-XIII, and this is confirmed by kinetic experiments with haemolysates.[10] The amount of harderoporphyrin which can be isolated from haemolysate experiments with coproporphyrinogen-III is <10% of added substrate.[11] In contrast to previous findings,[2] however, both coproporphyrinogens-III and -IV are metabolised at similar overall rates to the corresponding protoporphyrins. Full details of these and related experiments will be published elsewhere; the synthesis of the tricarboxylic porphyrin derived from coproporphyrinogen-IV is in progress.

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